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Comparative Study of a Modified Competitive RT-PCR and Amplicor HCV Monitor Assays for Quantitation of Hepatitis C Virus RNA in Serum

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A modified competitive RT-PCR (mcRT-PCR) to measure HCV RNA in serum and the Amplicor HCV Monitor assay were compared. For mcRT-PCR, the RNA extracted was retrotranscribed and coamplified in one step with a known amount of a DNA internal control (IC). Digoxigenin-labeled amplified products were hybridized to specific HCV DNA and IC-DNA probes and quantified by colorimetry. HCV RNA concentration was calculated by plotting the ratio of HCV/IC ODs against a calibration curve. Multiple samples were analyzed in the same round and tedious titration of each sample with a competitor was unnecessary. The mcRT-PCR assay was linear from 6×10^3 to 6×10^7 copies/ml, whereas Amplicor was linear up to $1–2 \times 10^6$ copies/ml. HCV RNA was measured in samples from 75 carriers. There was agreement between both methods in type 1 infections but not in type 2 or type 3 infections, in which the values measured by Amplicor were, on average, 15 times lower than those measured by the mcRT-PCR. HCV RNA measured by Amplicor was higher in type 1 infections than in type 2 or 3 infections, but no differences were found when viral load was assessed by mcRT-PCR. The binding efficiency of the Amplicor-probe was greater for type 1 than for types 2 or 3, suggesting Amplicor underestimates the viral load in the latter types. In contrast, the mcRT-PCR is not affected by genotype-related variation of HCV. This study suggests that mcRT-PCR assay is reliable for sensitive and accurate measurement of HCV RNA over a broad range of values independently of the HCV genotype. ***J. Med. Virol. 58:35–43, 1999.***

KEY WORDS: HCV viral load; quantitative PCR; HCV genotypes

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide [Alter et al., 1989, 1992; Bruix et al., 1989; Sánchez-Tapias et al., 1990]. The spectrum of liver lesions associated with chronic HCV infection ranges from minimal liver lesions to severe chronic hepatitis, cirrhosis, and hepatocellular carcinoma.

Quantitative measurement of the concentration of RNA of HCV (HCV RNA) is gaining clinical relevance because high levels of viremia have been associated with increased risk of vertical transmission of HCV [Ohto et al., 1994; Zanetti et al., 1995] and with poor response to interferon therapy in patients with chronic infection [Hagiwara et al., 1993b; Kobayashi et al. 1993; Lau et al., 1993; Hino et al., 1994; Zein et al., 1996]. In addition, studies about the relation between the level of HCV viremia with the histological severity of the liver disease [Hagiwara et al., 1993a; Ishiyama et al., 1993; Lau et al., 1993; Gretch et al., 1994; Ravaggi et al., 1995] or with the infecting HCV genotype [Berger et al., 1996; Ravaggi et al., 1996; Smith et al., 1996; Zeuzem et al., 1996] have produced conflicting results. Discrepancies may be due to lack of standardization of laboratory methods available currently to quantify HCV RNA concentration in serum.

Measurement of HCV RNA concentration in serum was carried out previously by RT-PCR amplification of diluted specimens to end-point titration [Simmonds et al., 1990]. More recently, competitive RT-PCR techniques have been introduced to this purpose [Becker-Andre and Hahlbrock, 1989; Hagiwara et al., 1993a].

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Quantitation of HCV RNA by competitive PCR relies on amplification of viral molecules in the presence of a DNA or RNA competitor molecule, which is coamplified with the viral target but can be easily identified in the detection steps. In competitive RT-PCR, the amount of amplicons derived from native viral RNA and from the competitive template are compared. However, this method is time consuming because multiple reactions of PCR must be carried out for each sample. There are two commercially available tests to measure the level of HCV RNA in blood: the branched DNA (bDNA) assay [Alter et al., 1995] of Chiron Corporation (Emeryville, CA) and the Amplicor HCV Monitor assay [Miskovsky et al., 1996] of Roche Diagnostic Systems (Branchburg, NJ), which are now widely used for quantitative measurement of HCV RNA in serum.

A modified competitive RT-PCR (mcRT-PCR) assay has been developed [Olmedo et al., 1996]. In this method, the concentration of HCV RNA present in each sample is calculated by interpolating the ratio of HCV to competitor internal control (IC) coamplified products on a calibration curve. This approach allows analysis of multiple samples in the same round because only one reaction per sample is necessary.

In the present study, the performance of the Roche Amplicor HCV Monitor test and the mcRT-PCR assay developed in house were compared. In addition, the relationship between viremia levels, as estimated by each method, HCV genotype, and response to INF therapy were investigated.

MATERIALS AND METHODS

Clinical Specimens

Serum samples were obtained from 75 patients with biopsy-confirmed chronic hepatitis C who were referred to the Liver Unit of our center for treatment. Other causes of chronic liver disease were excluded and all the patients received a standard course of alpha-interferon therapy.

HCV Genotyping

The genotype of HCV was determined by nested PCR with type-specific primers targeting the core region of HCV according to the procedure described by Okamoto et al. [Okamoto et al., 1992] with some modifications [Forns et al., 1996]. Genotypes were confirmed by restriction fragment length polymorphism analysis according to the procedure described by Davidson et al. [1995].

In-House Modified Competitive RT-PCR Assay

Five standard controls containing 6×10^7 to 6×10^3 HCV genome molecules/ml (measured by Amplicor Monitor Assay), one positive and two negative control sera, and serum samples under test were processed in the same round in microcentrifuge tubes. Tubes were spiked with a constant amount of IC. After retrotranscription and amplification, coamplified HCV and IC products were hybridized separately to specific capture probes and detected by colorimetry in microtiter plates. The HCV/IC ratio of the colorimetric signal produced by each amplified product was calculated for every sample. Quantitation of HCV RNA in samples was obtained by interpolating HCV/IC ratio values on a calibration curve, which was constructed with ratio values from controls containing a known amount of HCV RNA.

HCV RNA was extracted from 140 μl of serum using commercially available columns (QIAamp HCV Kit; QIAGEN, Germany) according to the manufacturer's specifications. Retrotransciption and PCR amplification were performed in a single step. The reaction was carried out in a 100-μl volume of a solution containing 25 μl of extracted RNA, 10-mmol/L Tris-HCl (pH 8.3), 50-mmol/L KCl, 1.5-mmol/L $MgCl_2$, 200-mmol/L dATP, 200-mmol/L dCTP, 200-mmol/L dGTP, 190-mmol/L dTTP, 10-mmol/L digoxigenin-labeled dUTP (Boehringer Mannheim, Mannheim, Germany), 1 mmol/L of primers derived from 5′NCR of HCV [Garson et al., 1990] (sense NCR3: 5′-CCACCATAGATCTCTCCCCTGT-3′; antisense NCR4: 5′-CACTCTCGAGCACCCTATCAGGCAGT-3′), 20-U RNasin (Promega, Madison, WI), 5-U AMV reverse transcriptase (Promega), and 2.5-U Taq polymerase (Gibco-BRL, Gaithersburg, MD). A constant amount of IC (10,000 molecules) was added to the reaction mixture. RT was performed at 42°C for 30 min and was followed by 40 cycles of PCR amplification (94°C for 30 sec; 50°C for 30 sec; 72°C for 1 min) with an additional extension step at 72°C for 5 min. Reactions were carried out in a 480 Perkin-Elmer Cetus Thermal cycler.

Kwock and Higuchi's [1989] guidelines were strictly followed to avoid PCR contamination, except that different pipette sets and aerosol-resistant tips instead of positive displacement pipettes were used in each separate area of the laboratory

Digoxigenin-labeled PCR products were detected with commercially available reagents (PCR ELISA DIG Detection kit, Boehringer Mannheim) according to the manufacturer's specifications. Digoxigenin-labeled PCR products (10 μl) were denatured and hybridized separately to biotinylated probes (7.5 pmol/ml) specific for HCV (FX1: bio-5′-GTGGTCTGCGGAACCGGTGAGT-3), which was selected after alignment of 314 5′NCR HCV sequences of genotypes 1 to 6 [Smith et al., 1995] and for IC. Absorbances were read at 405 nm and 450 nm. Values were expressed in OD_{405}, but for samples or standard controls which gave an OD_{405} value exceeding the linear reading capability of the photometer, the equivalent OD_{405} was calculated as follows:

$$\text{equivalent } OD_{405} = K \times OD_{450}$$

where K is the ratio OD_{405}/OD_{450} on any standard or sample with an OD_{405} value between 1.3 and 1.9.

Synthesis and Cloning of the HCV Internal Competitor

A neutral heterologous DNA fragment was amplified by PCR using a pair of composite primers. These prim-

ers were synthesized by addition of 5′NCR HCV specific sequences (NCR3 and NCR4) at the 5′ end of 20mer primers hybridizing to neutral DNA. A second amplification was carried out using HCV specific primers in order to obtain a DNA fragment that incorporated specific HCV sequences at the 5′ ends and can therefore be used as a competitive internal control.

The DNA fragment was separated by electrophoresis in low-melting agarose, excised from the gel and purified by Wizard PCR Preps DNA Purification System (Promega). Purified DNA was ligated in the pGEM-T plasmid vector (pGEM-T Vector Systems, Promega). Upon transformation of *Escherichia coli* JM109 competent cells, bacteria from one colony were grown in Brain Heart medium with 60 mg/ml of ampicillin and large amounts of the recombinant plasmid were recovered using QIAEX kits (QIAGEN). Plasmids were quantitated carefully by spectrophotometric analysis and used as IC in the in-house mcRT-PCR method. Amplification of IC with primers NCR3 and NCR4 resulted in a fragment of 600 bp with a G + C content similar to that in amplificates from pure HCV templates.

Amplicor HCV Monitor Assay

Quantitation of RNA-HCV by Amplicor HCV Monitor test (Roche Diagnostic Systems) was carried out according to manufacturer's instructions.

Statistical Analysis

Means of quantitative variables were compared by the Student's *t*-test. The relation between serum HCV RNA concentration measured by Amplicor HCV Monitor and by in-house–modified competitive RT-PCR was studied by linear regression analysis. Agreement between the two methods of quantification was assessed as recommended by Bland and Altman [1986]. Differences in qualitative variables were analyzed by the chi-square test. A P value of <0.05 was considered significant.

RESULTS

Characteristics of Patients' Population

The main demographic, clinical, histopathological, and virological features as well as the results of interferon therapy are shown in Table I. Among the 75 patients studied, 49 (65.3%) were infected by HCV type 1 (subtype 1a in 3 and subtype 1b in 46), 9 (12%) by HCV type 2 (subtype 2a in 8 and subtype 2b in 1), and 17 (22.6%) by HCV type 3 (subtype 3a in all).

TABLE I. Characteristics of Patients

Age (mean ± standard deviation)	46.5 ± 14.0
Sex (male/female)	47/28
Source of infection:	
Blood transfusion	25
Intravenous drug user	10
Unknown	40
Aspartate aminotransferase (IU/l)[a]	93.5 ± 56.3
Alanine aminotransferase (IU/l)[a]	160.1 ± 109.9
Liver histology	
Mild chronic hepatitis	28
Moderate chronic hepatitis	33
Severe chronic hepatitis	10
Liver cirrhosis	4
HCV genotype	
Genotype 1a	3
Genotype 1b	46
Genotype 2a	8
Genotype 2b	1
Genotype 3a	17
Response to interferon therapy	
Sustained response	10
Transient response	25
No response	39

[a]Expressed as mean ± SD.

Comparison of Amplicor HCV Monitor and mcRT-PCR

HCV RNA concentration was determined by both assays in replicates of a serum sample containing 6×10^5 copies/ml of HCV genome in 10 independent batches. The interassay coefficient of variation (CV) was 37% for Amplicor HCV Monitor (all batches were performed using the same lot of reagents) and 34% for mcRT-PCR.

The range of quantitation of the two methods was explored by testing serial dilutions of a serum sample with a high concentration of HCV RNA (Fig. 1). Linearity of mcRT-PCR assay was verified at least in the range of the standard curve (6×10^7 to 6×10^3 copies/ml). Amplicor HCV Monitor method was linear up to $1–2 \times 10^6$ copies/ml only and higher concentrations of HCV RNA were underestimated by this method.

The correlations between the log of HCV RNA serum concentration measured by the two methods in sera infected with HCV type 1 and HCV types 2 and 3 are shown in Figures 2A and 2B, respectively. In type 1 sera, the regression coefficient was 0.854 ($P < 0.0001$) and the mean value of HCV RNA levels measured by both methods was similar (Table II). A good correlation was also observed between the results of both methods in sera infected with HCV types 2 and 3 (regression coefficient = 0.725, $P < 0.0001$). However, the concentration of HCV RNA measured by Amplicor in type 2– and type 3–infected sera was significantly lower in comparison with values measured by mRT-PCR (Table II).

To assess the agreement between the two methods, the arithmetic mean of the logarithmic transformation of HCV RNA values measured in the every sample by each assay was plotted against their difference (mRT-PCR – Amplicor) according to Bland and Altman [1986] (Fig. 3). The overall mean of the difference in sera from patients infected by HCV type 1 was close to zero (–0.003), indicating good agreement between both assays in these patients. However, the frequency distribution of these data was not normal but bimodal. Thus, sera with HCV concentration lower and higher than 6 log (according to mcRT-PCR assay) were analyzed separately. As shown in Figure 3A, the mean of the difference in low-concentration sera was –0.1 and the 95% limits of agreement (mean of difference ± 2 × standard deviation) were –0.72 and +0.44 (1.1 orders of

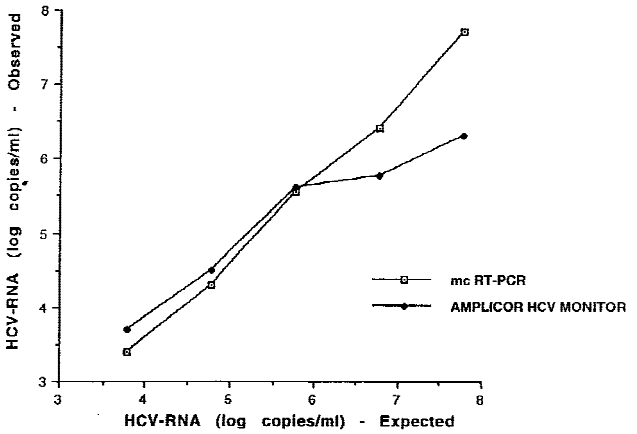

Fig. 1. Range of quantification of HCV RNA of mcRT-PCR and Amplicor HCV Monitor assays.

magnitude). In high-concentration sera, the mean of the difference was 0.4 (Fig. 3B). This indicates that Amplicor may underestimate HCV RNA concentration in highly viremic sera, as expected from the shorter linear range of this method. However, scatter of the data obtained in highly viremic sera in which 95% of values were between −0.25 and +1.03 (1.2 orders of magnitude) was similar to that observed in low viremic sera.

In contrast, important disagreement between the two methods was noted when measuring HCV RNA concentration in sera from patients infected by HCV type 2 and type 3. The mean of the difference in this group was 1.2 (Fig. 3C), indicating that the concentration of HCV RNA measured by Amplicor was, on average, 1.2 logs lower than the concentration measured by mcRT-PCR. In addition, in type 2 and type 3 sera, 95% of the data were scattered between −0.22 and +2.58, a range of 2.8 orders of magnitude, which would be unacceptable for clinical purposes.

HCV RNA Concentration and HCV Genotype

As shown in Table II, the concentration of HCV RNA in serum was higher in patients infected with HCV type 1 than in those infected with type 2 ($P < 0.05$) or type 3 ($P < 0.01$) when measured by Amplicor (Fig. 4A), but no significant differences between genotypes were observed when tested by mcRT-PCR (Fig. 4B). In order to investigate the cause of this difference between the two methods, the efficiency of the probes used in this study to hybridize to HCV DNA amplified from different HCV genotypes was analyzed. Serial fivefold dilutions of a known amount of purified HCV amplicons from three specimens of each genotype were hybridized to HCV DNA detecting probes from Amplicor and from mcRT-PCR. The signal obtained after hybridization with the Amplicor probe was stronger in type 1 (61.5 ± 20 OD) than in type 2 (2.3 ± 0.9 OD) or type 3 (4.7 ± 4.7 OD) samples. In contrast, no differences were found in the binding efficiency of FX-1 probe of mRT-PCR to the genotypic variants of HCV under study.

Relationship Between HCV RNA Serum Concentration and Response to Interferon Treatment

Sustained response was more frequent ($P = 0.004$) in patients infected by HCV type 2 (2/9) and type 3 (6/17) than in those infected by type 1 (2/49). Pretherapy HCV RNA serum concentration measured by Amplicor was significantly lower ($P < 0.05$) in sustained responders (18,400 ± 24,390 copies/ml) than in nonresponders (500,130 ± 781,990 copies/ml) (Table III). Serum HCV RNA concentration was lower than 70,000 copies/ml in sustained responders (Fig. 4A).

When measured by mcRT-PCR, pretherapy serum HCV RNA was lower in sustained responders (752,620 ± 1,481,610 copies/ml) than in nonresponders (892,930 ± 1,286,750 copies/ml), but the difference was not significant ($P = 0.7$) (Table III). In fact, three of six sustained responders infected with type 3 HVC appeared to be highly viremic when HCV RNA was measured by mRT-PCR (Fig. 4B).

DISCUSSION

Classical competitive PCR is a sensitive and accurate procedure for measurement of HCV RNA concentration, but it is tedious because multiple reactions of PCR must be carried out to titrate each sample [Hagiwara et al., 1993a; Gretch et al., 1995]. Another disadvantage of the titration methods is that the dynamic range and accuracy are mutually exclusive, unless an extremely large number of assays are performed with each sample. Recently, a modified competitive PCR to measure target DNA has been reported [Jalava et al., 1993; Lehtovaara et al., 1993; Zachar et al., 1993]. In this assay, a single amplification is sufficient because quantitation is achieved by comparison with a single set of standard controls. Consequently, multiple PCR reactions for each sample, as required in classical competitive PCR, are not necessary and multiple samples can be tested in one round. These principles were applied to develop a modified competitive RT-PCR (mcRT-PCR) to measure HCV RNA serum concentration in which DNA molecules are used as internal competitor.

In mcRT-PCR, intra-assay variation of the efficacy of amplification is compensated by incorporation to the reaction of an internal DNA standard. Moreover, addition of an internal standard makes it possible to extend the linear range of the assay when the amplification rate of the HCV RNA target reaches the plateau. In this later phase of PCR amplification, the amount of amplicons is not longer proportional to the starting amount of target molecules. However, when the amount of the target is high, amplification of the internal standard is inhibited by competition and the degree of inhibition is proportional to the initial amount of target molecules in the reaction. Consequently, in mcRT-PCR for HCV RNA, the final ratio between HCV and internal standard amplicons will be proportional to initial amount of HCV RNA molecules [Jalava et al., 1993].

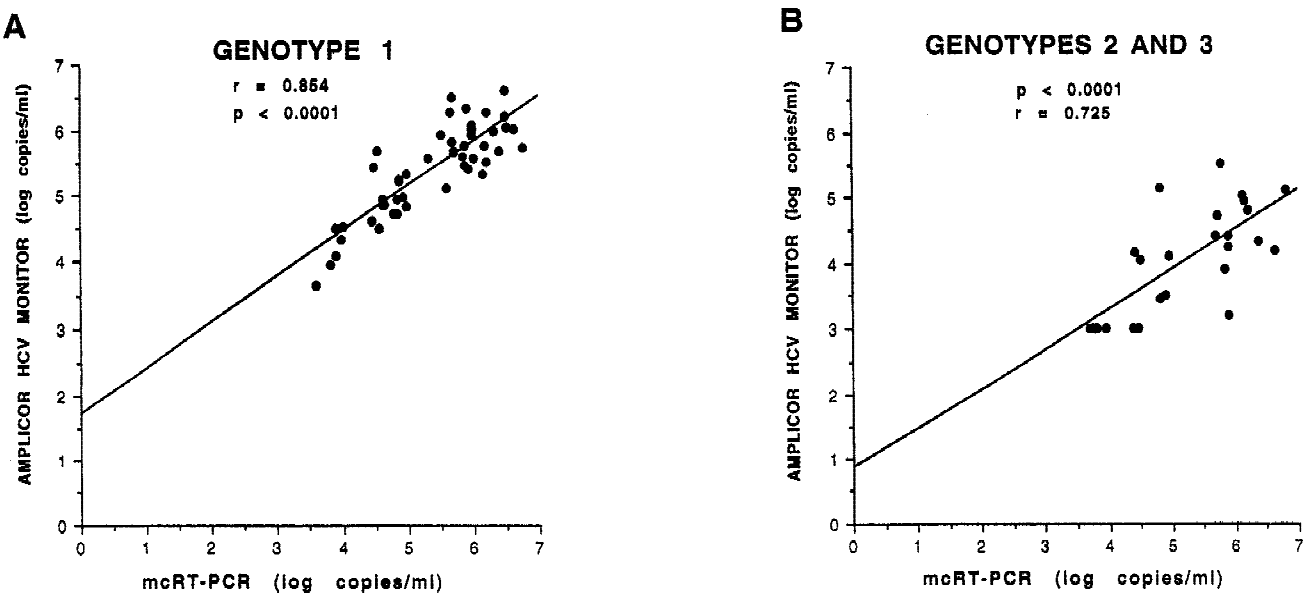

Fig. 2. Correlation between mcRT-PCR assay and Amplicor HCV Monitor assay for measure HCV RNA concentrations in sera from patients infected by genotype 1 (**A**) and from those infected by genotypes 2 and 3 (**B**). Levels are plotted as log copies/ml.

TABLE II. HCV RNA Levels in Relation to Infecting Genotype as Measured by Amplicor HCV Monitor and by mcRT-PCR Assays

	Genotype 1 (n = 49)	Genotype 2 (n = 9)	Genotype 3 (n = 17)
Amplicor HCV Monitor[a] (mean ± standard deviation)	627 ± 842	35 ± 52	43 ± 80
mcRT-PCR[a] (mean ± standard deviation)	844 ± 1187	365 ± 633	1140 ± 1753
P value	Not significant	<0.01	<0.001

[a]Values of RNA HCV are expressed in $\times 10^3$ copies/ml.

If the amplification efficiency of the competitive templates is identical, it would be theoretically possible to calculate the concentration of HCV RNA by multiplying the known initial amount of IC by the HCV/IC ratio measured after amplification, as in Amplicor assay. However, due to reannealing of PCR products, heteroduplex formation or other reasons, the amplification efficiency of each template may not be identical, leading to deviation of the slope of the experimental curve with respect to the theoretically expected curve. This problem may be circumvented by a interpolating HCV/IC ratio values on a standard curve drawn with a series of appropriate controls [Lehtovaara et al., 1993; Zachar et al., 1993; Mayerat et al., 1996].

In Amplicor assay, an internal RNA standard is added to the sample prior to extraction and retrotranscription, whereas in the mcRT-PCR described above the internal standard consists of a DNA molecule that is added to extracted RNA. Use of DNA molecules as internal standards may simplify quantitation of RNA by competitive PCR because, in contrast with RNA molecules, DNA molecules can be obtained easily in large amounts and are stable for prolonged periods of time. However, losses of HCV RNA during extraction or variations of the efficiency of retrotranscription can not be controlled when using internal DNA standards and underestimation of HCV RNA may occur. However, in the in-house mcRT-PCR procedure described above, underestimation was prevented by interpolating the observed values on a standard curve. This curve was drawn using the HCV RNA/IC ratio values measured in serial dilutions of a serum sample that contained a known amount of HCV RNA that were extracted, retrotranscribed, and amplified exactly as serum samples under study, in order to balance for potential losses during extraction. The good correlation of HCV RNA values measured by mcRT-PCR and Amplicor in sera containing genotype 1b suggests that use of an internal DNA molecule, under the conditions described above, did not prevent accurate quantitation of serum HCV RNA.

In addition, the interassay variations of mcRT-PCR and Amplicor were similar and comparable to that observed after quantitation by other competitive PCR procedures that use RNA molecules as internal standard [Mayerat et al., 1996; Roth et al., 1996; Pawlotsky, 1997], suggesting that use of DNA internal standard does not impair the reproducibility of quantitation.

mcRT-PCR appears to be suitable for quantitation of

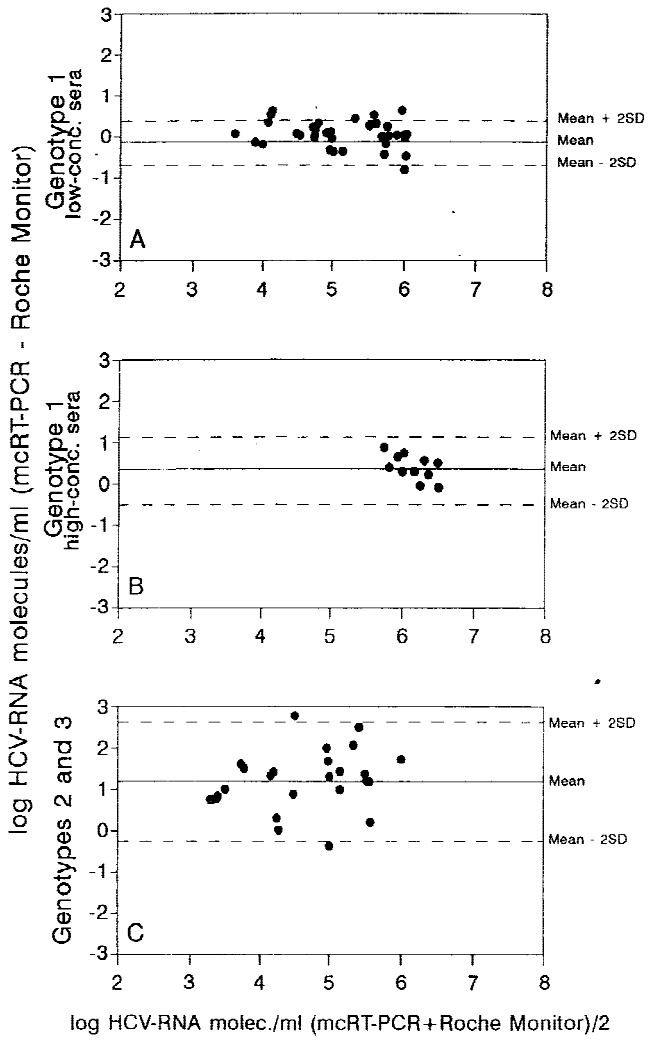

Fig. 3. Difference against the mean of logarithmic transformed HCV RNA concentrations measured by mcRT-PCR and Amplicor HCV Monitor assays: low-concentration sera from patients infected by genotype 1 (**A**), high-concentration sera from patients infected by genotype 1 (**B**), and sera from patients infected by genotypes 2 and 3 (**C**).

serum HCV RNA in the majority of patients with chronic hepatitis, since this assay was linear at least between 6×10^3 and 6×10^7 copies/ml. In contrast, Amplicor was linear up to $1–2 \times 10^6$ copies/ml and, therefore, repeated testing of diluted samples is necessary for accurate measurement of HCV RNA in samples with high viral load.

A significant agreement was found between both methods in sera from patients infected with HCV type 1. However, important differences were observed when measuring HCV RNA concentration in sera infected with HCV types 2 and 3. Values obtained with Amplicor were, on average, 15 times lower than those measured by mcRT-PCR. In the absence of a gold standard, it is difficult to ascertain which of the two methods is correct. In previous studies using Amplicor HCV Monitor, bDNA assay of first-generation or competitive PCR, the level of viremia was higher in genotype 1 than in genotype 2 and genotype 3 infections [Mahaney et al., 1994; Chan et al., 1995; Berger et al., 1996]. The 5′NCR was the region of HCV genome selected for quantitation in these assays because it is highly conserved. However, in the last few years considerable variation in the sequence of the 5′NCR of different HCV genotypes had been identified with important implications for the selection of primers and probes [Smith et al., 1995]. Inappropriate selection of primers or probes can lead to differences in the sensitivity of the assays to detect HCV RNA of different genotypes. In fact, it has recently been demonstrated that HCV RNA serum level in patients infected with HCV types 2 and 3 is underestimated by first-generation bDNA assay [Lau et al., 1995; Detmer et al., 1996]. When appropriate correction factors were applied on bDNA values or a second-generation bDNA assay was used, no significant differences were found in the levels of viremia between HCV types 1, 2, and 3 [Smith et al., 1995; Lau et al., 1996; Hawkins et al., 1997]. In addition, it has recently been shown that the sensitivity of Amplicor for genotype 2 and 3 transcripts was much lower than for genotype 1 [Hawkins et al., 1997]. Moreover, in the current study, the binding efficiency of the Amplicor detecting probe was less for genotypes 2 and 3 than for genotype 1. These findings suggest strongly that Amplicor underestimates the viral load in sera infected with genotypes 2 and 3. Mismatches between the PCR primers and the target HCV had been suggested as the origin of the reduced sensitivity of Roche assay for these genotypes [Hawkins et al., 1997]. Our results suggest that the efficiency of hybridization for the different genotypes could be an important factor also. Nevertheless, the inferior efficiency of the Amplicor probe for genotypes 2 and 3 cannot be attributable to variation in the target sequence because it is highly conserved [O'Brien et al., 1997], but differences in the secondary structure of the 5′NCR between HCV genotypes could be involved.

Several reports have shown consistently high viremia and genotype 1b infections are associated with a poor response to interferon therapy [Hagiwara et al., 1993b; Kobayashi et al., 1993; Lau et al., 1993; Hino et al., 1994; Zein et al., 1996]. However, the level of viremia was often measured by laboratory methods that may underestimate HCV RNA serum concentration in type 2 and type 3 HCV infections. Therefore, it is unclear at present which of these factors, the level of viremia or the infecting HCV genotype, is more important as a predictor of a favorable response to therapy. To solve this issue, therapeutic trials aimed at evaluating the efficiency of interferon therapy in chronic hepatitis C should incorporate measurement of HCV RNA serum concentration by methods that are not affected by genotype variability.

Other methods for HCV RNA measurement had been reported, such as limiting dilution method, multicyclic RT-PCR, and bDNA. The dilution RT-PCR is a semiquantitative assay and it is not an accurate method to

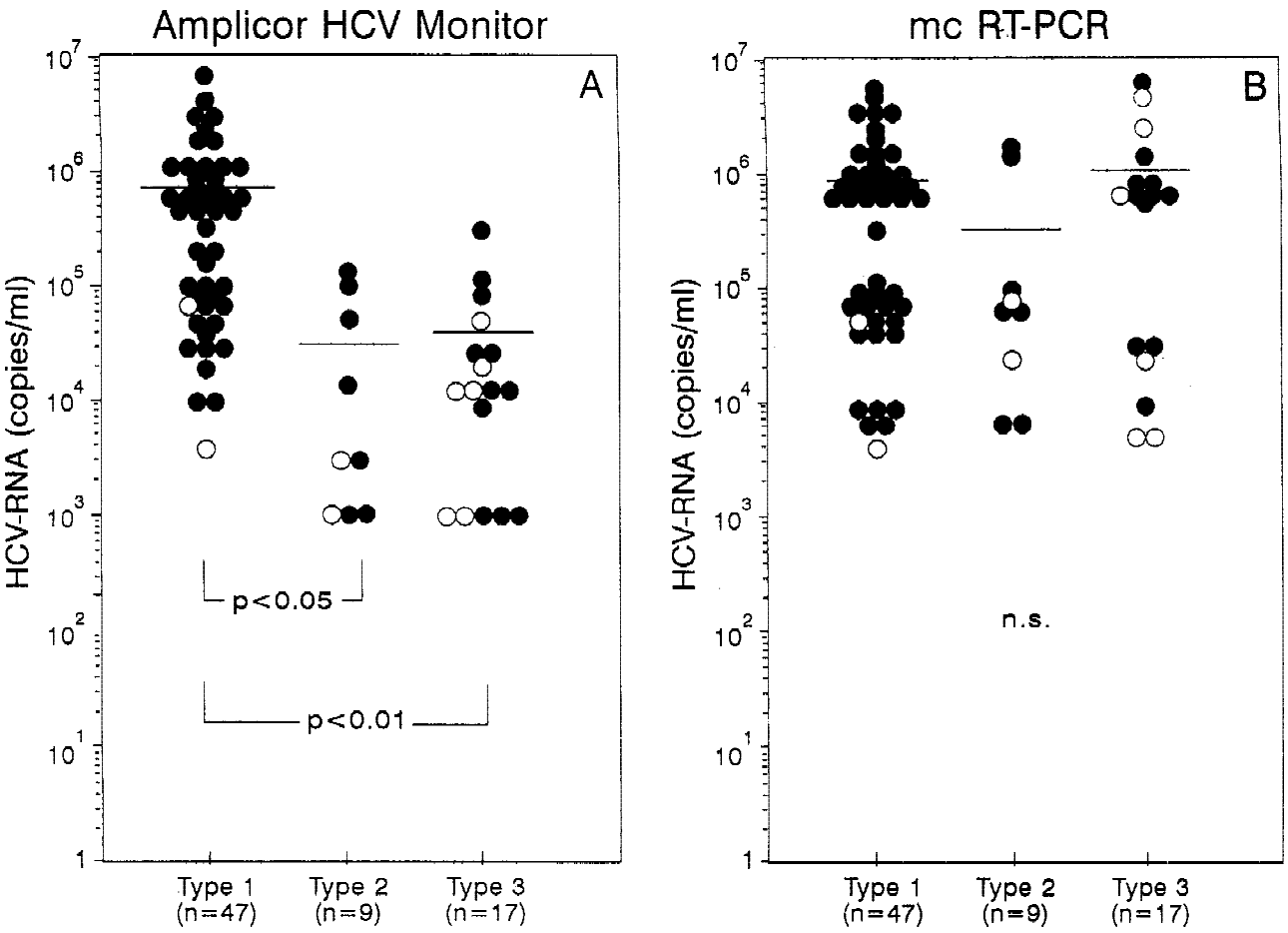

Fig. 4. Amounts of circulating HCV RNA in relation to the infecting genotype in patients with chronic hepatitis C before interferon therapy, measured by Amplicor HCV Monitor assay (**A**) and by mcRT-PCR assay (**B**). Open and close circles represent the HCV RNA concentration from responders and from nonresponders, respectively. Median values for each genotype are indicated by short solid lines.

TABLE III. Levels of HCV RNA in Relation to Response to Interferon Therapy as Measured by Amplicor HCV Monitor and by mcRT-PCR Assays

	mcRT-PCR[a] (mean ± SD)	Amplicor HCV Monitor[a] (mean ± SD)
Responders (n = 10)	752 ± 1481	18.4 ± 24.4
Nonresponders (n = 65)	892 ± 1286	500 ± 781
P value	0.7	<0.05

[a]Values of RNA HCV are expressed in $\times 10^3$ copies/ml.

measure HCV RNA concentration. In addition, similar to competitive RT-PCR, the dilution method is tedious and expensive because several reactions can be carried out for each sample. Multicyclic RT-PCR is more accurate but it has two disadvantages. First, it is tedious because aliquots of the amplified products must be removed and saved for dot blot hybridization several times during the amplification process. Second, variations in the efficiency of amplification because of the presence of inhibitors can impair the accuracy of the measurement. The branched DNA probe assay of second generation is a simple, accurate, and reliable method to measure HCV RNA concentration of all HCV genotypes. However, the bDNA assay is less sensitive than PCR-based methods and, as Amplicor assay, it is very expensive.

The mcRT-PCR described above is a cost-effective, sensitive, and reproducible assay to measure HCV RNA concentration in serum over a broad range of values and is not affected by variability between major HCV genotypes. The simplicity of the procedure (rapid extraction, one-step RT-PCR, and colorimetric detection on microplates) allows mcRT-PCR assay to be easily performed in conventional clinical laboratories.

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